AMENDMENTS TO THE CLAIMS

Please amend the claims as follows:

1-50. (Cancelled)

51. (Withdrawn) Screening method of molecules capable of generating the alteration of a target intracellular parameter, said alteration being the variation in the concentration of a second messenger that is a cyclic nucleotide, said alteration being converted into a proportional variation in the intracellular concentration of the Ca2+ ion, detected by means of a Ca2+-sensitive recombinant aequorin probe, comprising the following phases:

a) construction of an expression vector containing the fusion protein sequence encoding said probe, said sequence being characterized in that it comprises the Ca2+-sensitive recombinant aequorin encoding sequence, condensed together with at least one signal sequence;

b) transfection of at least one of a mammalian cell line with said vector containing the Ca2+-sensitive recombinant aequorin probe, said cell line being previously engineered so as to express an heterologous chimeric receptor being characterized in that it has the intracellular portion of a receptor coupled with variations in the concentration of calcium and the extra-cellular portion of a receptor coupled with the production of cyclic nucleotides;

c) activation of said Ca2+-sensitive aequorin probe by the addition of a prosthetic group to the cellular line expressing said recombinant protein probe;

d) administration of the molecule to be tested to the cellular line expressing said recombinant protein probe;

e) detection of the emission of photons on the part of the Ca2+-sensitive aequorin probe expressed in the cellular line and evaluate the amount of activation or inhibition exerted by the tested molecule, on the basis of a ratio between the cps value obtained and the maximum value of cps registered under conditions of maximum stimulation of the cellular line.

52. (Withdrawn) Screening method according to claim 51, wherein said prosthetic group is celentherazine.

53. (Withdrawn) Screening method according to claim 51, wherein said cyclic nucleotide is c-AMP.

54. (Withdrawn) Screening method according to anyone of the claim 51, wherein said signal sequence directs the Ca2+-sensitive recombinant aequorin probe to a cellular compartment, said probe being the fusion protein mt-aequorin (mt-AEQ).

55. (Currently Amended) Screening method of molecules capable of generating the alteration of a target intracellular parameter, said alteration being the translocation from cytoplasm to the membrane of a cellular effector, said translocation being correlated to the different intracellular concentration of the Ca2+ ion between cytoplasm and submembrane area, detected by means of a Ca2+-sensitive recombinant aequorin probe, comprising the following phases:

a) construction of an expression vector containing the fusion protein sequence encoding said probe, said sequence being characterized in that it comprises sequences encoding at least one Ca2+-sensitive recombinant aequorin encoding sequence, condensed together with at least one cellular effector ~~and/or a signal sequence~~, or a combination of at least one cellular effector and at least one signal sequence;

b) transfection of at least one mammalian cell line with said vector containing the Ca2+-sensitive recombinant protein probe;

c) activation of said Ca2+-sensitive photo-protein by the addition of a prosthetic group to the cellular line expressing said recombinant protein probe;

d) administration of the molecule to be tested to the cellular line expressing said recombinant protein probe;

e) detection of the emission of photons on the part of the Ca2+-sensitive photo-protein expressed in the cellular line and evaluate the amount of activation or inhibition exerted by the tested molecule, on the basis of a ratio between the cps value obtained and the maximum value of cps registered under conditions of maximum stimulation of the cellular line.

56. (Previously Presented) Screening method according to claim 55, wherein said prosthetic group is celentherazine.

57. (Previously Presented) Screening method according to claim 55, wherein said cellular effector is a regulating protein.

58. (Previously Presented) Screening method according to claim 57, wherein said regulating protein is selected from the group which comprises protein-kinase, phosphatase, adenylate cyclase, proteins that link plasmatic membrane receptors, proteins that interact with plasmatic membrane channels, proteins that interact with plasmatic membrane lipids.

59. (Previously Presented) Screening method according to claim 55, wherein said signal sequence directs the Ca2+-sensitive recombinant aequorin probe to a cellular compartment.

60. (Withdrawn) Screening method according to claim 55, wherein said probe is a fusion protein selected from the group that consists of PKC-aequorin (PKC-AEQ) and shc-aequorin (shc-AEQ).

61. (Withdrawn) Screening method according to claim 60, wherein the PKC-aequorin is selected from the group comprising PKC beta-aequorin, PKC delta-aequorin, PKC epsilon-aequorin, PKC zeta-aequorin, PKC gamma-aequorin, PKC alpha-aequorin, PKC-lambda-aequorin, PKC theta-aequorin, PKC eta-aequorin.

62. (Withdrawn) Screening method according to claim 60, wherein the shc-aequorin is selected from the group consisting of p66shc-aequorin, p46shc-aequorin, p52shc-aequorin.

63. (Previously Presented) Screening method according to claim 55, wherein the expression vector of phase a) is a eukaryotic vector.

64. (Currently Amended) Screening method according to claim 55, wherein said at least one mammal cellular line of phase b) is previously engineered so as to express a heterologous ~~native~~ or chimeric protein.

65. (Previously Presented) Screening method according to claim 64, wherein said heterologous protein is selected from the group which consists of a receptor, an enzyme, a ionic channel and a cellular effector.

66. (Previously Presented) Screening method according to claim 64, wherein said chimeric protein is a chimeric receptor.

67. (Withdrawn) Screening method according to claim 65, wherein said ionic channel is selected from the group which comprises voltage-dependent Ca2+ channels and Ca2+ channel receptors.

68. (Withdrawn) Screening method according to claim 65, wherein said cellular effector is a regulating protein selected from the group which comprises protein-kinase, phosphatase, adenylate cyclase, proteins that links plasmatic membrane receptors, proteins that interacts with plasmatic membrane channels, proteins that interacts with plasmatic membrane lipids.

69. (Currently Amended) Screening method according to claim 65, wherein said ~~cellular~~ receptor is a cell membrane receptor selected from the group which comprises receptors coupled with G proteins, receptors with an enzymatic activity, channel receptors.

70. (Withdrawn) A Ca2+-sensitive recombinant fusion protein probe, characterized in that it comprises the sequence encoding at least one Ca2+-sensitive recombinant aequorin encoding sequence, condensed together with at least one cellular effector and/or a signal sequence, said cellular effector being a regulating protein selected from a protein-kinase and a protein that links plasmatic membrane receptors.

71. (Withdrawn) Probe according to claim 70, wherein said protein-kinase is a protein kinase C (PKC).

72. (Withdrawn) Probe according to claim 71, wherein said PKC-aequorin is selected from the group which comprises PKC beta-aequorin (PCK beta: rif. M13975), PKC delta-aequorin (PCK delta: rif. M18330), PKC epsilon-aequorin (PCK epsilon: rif. AF028009), PKC zeta-aequorin (PCK zeta: rif. M18332), PKC gamma-aequorin, PKC alpha-aequorin (PCK alfa: rif. M13973), PKC-lambda-aequorin, PKC theta-aequorin (PCK theta: rif. L07032), PKC eta-aequorin.

73. (Withdrawn) Probe according to claim 70, wherein said protein that links plasmatic membrane receptors is an adaptor protein.

74. (Withdrawn) Probe according to claim 73, wherein said adaptor protein belongs to the shc family.

75. (Withdrawn) Probe according to claim 74, wherein the protein is selected from the group comprising p46shc, p52shc and p66shc.

76. (Withdrawn) Probe according to claim 70, wherein said cell membrane receptor is selected from the group which comprises receptors coupled with G proteins, receptors with an enzymatic activity, channel receptors.

77. (Withdrawn) Probe according to claim 70, wherein said signal sequence directs the Ca2+-sensitive photo-protein, preferably aequorin, towards a cellular compartment.

78. (Withdrawn) Use of the Ca2+-sensitive recombinant fusion protein probe as defined in claim 70, for the screening of molecules capable of generating the alteration of an intracellular parameter, said alteration being the translocation from cytoplasm to the membrane of a cellular effector, said translocation being correlated to the different intracellular concentration of the Ca2+ ion between cytoplasm and submembrane area.

79. (Withdrawn) Use according to claim 78, wherein said cellular effector is selected from the group which consists of ionic channel, regulating protein, cell membrane receptor.

80. (Withdrawn) Use according to claim 79, wherein said ionic channel is selected from the group which comprises voltage-dependent Ca2+ channels and Ca2+ channel receptors.

81. (Withdrawn) Use according to claim 79, wherein said regulating protein is selected from the group which comprises protein-kinase, phosphatase, adenylate cyclase, proteins that links plasmatic membrane receptors, proteins that interacts with plasmatic membrane channels, proteins that interacts with plasmatic membrane lipids.

82. (Withdrawn) Use according to claim 79, wherein said cell membrane receptor is selected from the group which comprises receptors coupled with G proteins, receptors with an enzymatic activity, channel receptors.

83. (Withdrawn) Screening method of molecules capable of generating the alteration of a target intracellular parameter, said alteration being the activation/inactivation of a cellular effector that is a protein kinase acting on Ca2+ ionic channels, said alteration being converted into a proportional variation in the intracellular concentration of the Ca2+ ion, detected by means of a Ca2+-sensitive recombinant aequorin probe, comprising the following phases:

a) construction of an expression vector containing the fusion protein sequence encoding said probe, said sequence being characterized in that it comprises the Ca2+-sensitive recombinant aequorin encoding sequence, condensed together with at least one signal sequence;

b) transfection of at least one of a mammalian cell line with said vector containing the Ca2+-sensitive recombinant aequorin probe;

c) activation of said Ca2+-sensitive aequorin probe by the addition of a prosthetic group to the cellular line expressing said recombinant protein probe;

d) administration of the molecule to be tested to the cellular line expressing said recombinant protein probe;

e) detection of the emission of photons on the part of the Ca2+-sensitive aequorin probe expressed in the cellular line and evaluate the amount of activation or inhibition exerted by the tested molecule, on the basis of a ratio between the cps value obtained and the maximum value of cps registered under conditions of maximum stimulation of the cellular line.

84. (Withdrawn) Screening method according to claim 83, wherein said prosthetic group is celentherazine.

85. (Withdrawn) Screening method according to claim 83, wherein said cellular effector is PKC.

86. (Withdrawn) Screening method according to claim 83, wherein said Ca2+ ionic channel is selected from the group which comprises voltage dependent Ca2+ channels and Ca2+ channel-receptors.

87. (Withdrawn) Screening method according to claim 86, wherein said Ca2+ channels are L type Ca2+ channels.

88. (Withdrawn) Screening method according to claim 83, wherein said signal sequence directs the Ca2+-sensitive recombinant aequorin probe to a cellular compartment.

89. (Withdrawn) Screening method according to claim 83, said probe being a fusion protein selected from the group which comprises SNAP aequorin (SNAP-AEQ), mt-aequorin (mt-AEQ) and cytosol aequorin (cyt-AEQ).

90. (Withdrawn) Screening method according to claim 83, wherein said at least one mammal cellular line of phase b) is previously engineered so as to express a heterologous native or chimeric protein.

91. (Withdrawn) Screening method according to claim 90, wherein said heterologous protein is selected from the group which consists of ionic channels.

92. (Withdrawn) Screening method according to claim 91, wherein said ionic channel is selected from the group which comprises voltage-dependent Ca2+ channels and Ca2+ channel receptors.

93. (Withdrawn) Screening method according to claim 92, wherein said Ca2+ channels are L type Ca2+ channels.

94. (New) Screening method according to claim 55, wherein said cellular effector is selected from the group consisting of protein kinases and protein adaptors.